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Melvin E. Tyson
Closeout Administrator
National Aeronautics and Space Administration
Code CWC-1/MT
Washington, DC 20546

Dear Mr. Tyson:

**Grant NAGW 3672**

Enclosed is the information requested

A. Final Property Inventory:
The following equipment was purchased on the grant:

1 Fast Protein Liquid Chromatography Column (Preparative) Mono S
1 HPLC Autosampler (Waters, Model 717)

The following is on loan from NASA (Kennedy Space Center, Bionetics):

1 Plant Growth Facility (for growing plants in space)

B. Final Technical Report: Enclosed

C. Final Patent/Invention Report:

Heyenga, G. and Lewis, N.G.: A Solid-state Nutrient Support System for Long-Term Plant Culture in Microgravity Conditions.

I trust everything is in order. If you have any questions, please do not hesitate to contact me.

Yours sincerely,

N. G. Lewis

Norman G. Lewis
Director

NGL:kfm
Enclosure
cc: T.K. Scott
NASA Scientific and Technical Information Facility

# Final Report: Plant Metabolism and Cell Wall formation in Space (Microgravity) and On Earth

by

Norman G. Lewis

## Introduction

A principal focus of our research is to precisely and systematically dissect the essentially unknown mechanism(s) of vascular plant cell wall assembly, particularly with respect to biosynthesis of their phenolic constituents (i.e., lignins and suberins), and how gravity impacts their formation. Assembly of these phenolic polymers is of particular interest, since it appears that elaboration of their biochemical pathways was essential for successful land adaptation. We are also greatly intrigued as to how the "absence" of gravity affects "normal" cell wall assembly mechanisms/metabolism.

The following three sections describe recent progress related to unraveling the fundamental biochemistry of plant phenolic biochemistry, and the effect of gravity on plant growth and development. The first area describes approaches in delineating the fundamental basis of secondary cell wall assembly mechanisms (ground-based studies), and the second examines the effect of microgravity proper (shuttle flight studies) on plant cell wall formation/metabolism.

**Progress:** The following discoveries were made: using cell cultures of *Pinus taeda* (loblolly pine), it has been possible to attain a cell line which can be induced to undergo a developmental-like transition from an unlignified, primary wall phase or stage to that of secondary wall (~$S_1$ deposition) lignification. Judicious carbon-13 labeling established that the lignin was a relatively high fidelity copy of a softwood gymnosperm lignin (Eberhardt, *et al.*, 1993; Lewis, 1994, 1993; Davin and Lewis, 1992). Since then, we have established that the cell cultures can be induced to undergo lignin synthesis via alteration of the osmoticum, and that lignin deposition proper can be totally inhibited by culturing the cells in the presence of $H_2O_2$ scavengers. This results in complete inhibition of lignification, while yet maintaining the capacity to synthesize the monolignols themselves, which are secreted into the "extracellular" medium (Nose *et al.*, 1995). This is the first instance where lignin synthesis, but not monolignol formation, can be fully separated. This discovery has several important ramifications: (1) it is now being used to study flux in the phenylpropanoid pathway, i.e., to ascertain the rate-limiting steps and how monomer composition is affected, as well as how "gravity perception" alters the monolignol composition/lignification response.

In a related manner, the ability to dissect the pathway to the lignins enabled us to address a long-standing enigma; namely, what is the fate of the ammonium ion released during active phenylpropanoid metabolism. This is an important question since *ca* 30 to 40% of all plant material is of phenylpropanoid origin, i.e., an equivalent amount of ammonium ion is released during its formation. By appropriate HPLC quantification, $^{15}$N-NMR, and GC-MS analyses, it was possible to establish that a new mechanism for nitrogen recycling was operative, i.e., the ammonium ion released was not made available for general amino acid formation/protein synthesis. Rather, it was rapidly metabolized back to glutamate, which then served as amino acid donor for regeneration of phenylalanine, i.e., an efficient mechanism of nitrogen recycling exists which now explains why organisms undergoing active phenylpropanoid metabolism (i.e., lignin synthesis) do not exhibit nitrogen deficiency (Van Heerden *et al.*, 1996).

Other associated phenylpropanoid pathways were also investigated, i.e., those leading to the suberins. Since suberization has long been proposed to be a physiological response forming a

lignin-like polymer (in terms of its phenolic constituents), we investigated whether its structure was in fact lignin or not. Identification of putative suberin fragments, and the application of solid-state NMR carbon-13 spectroscopy (using specific carbon-13 enriched precursors) established that the polymer was composed of hydroxycinnamic acids, rather than monolignols/lignin. Moreover, these hydroxycinnamates were linked to aliphatic constituents and/or amides. Thus, a clear distinction between the lignin and suberin pathways has now been made (Bernards and Lewis, 1995; Bernards *et al.*, 1995; Bernards and Lewis, 1992).

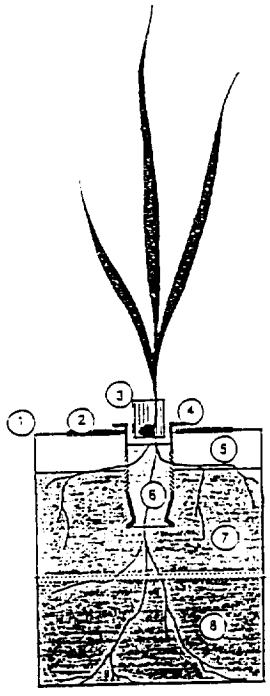
Another major discovery was made addressing the question of how lignin and lignan pathways, which use the same monolignol precursor, can be channeled into lignins (for structural support, gravitational realignment, etc.) and into lignans (for plant defense as biocides, antioxidants). This is an extremely important issue since it requires "direction" of the appropriate monolignol precursors into the appropriate pathway and, hence, the question of how their assembly mechanisms are orchestrated *in vivo*. In this regard, it was found that, in addition to peroxidase/laccase, another piece to the biochemical jigsaw puzzle was discovered. That is, we have found that a "specifier" protein controls the coupling modes (in preparation ) and, thus, the fate/direction of phenolic coupling; there is no known biochemical precedent to this discovery. [This result came from the systematic study of phenolic coupling mechanisms (Davin and Lewis, 1992; Katayama *et al.*, 1992; Davin *et al.*, 1992; Lewis *et al.*, 1992; Ozawa *et al.*, 1993).

**Significance:** It has long been known that lignins vary with cell type and species, and that gravitational corrections result in altered lignin compositions in the tissues so affected (e.g., a higher *p*-coumaryl alcohol content). Our recent findings now provide a means to systematically define how these compositional changes are regulated, i.e., what factors differentially control individual monomeric synthesis and how precise mechanisms of assembly are controlled in different cell types. A detailed understanding of such processes is essential if we are to define how lignin synthesis is regulated during both "normal" and "gravitational correctional" developmental processes.

## Space Shuttle Studies

Previous difficulties in growing plants in space have been partially overcome by development of a nutrient agar pack (Heyenga *et al.*, 1996) suitable for the uninterrupted growth/development of plants in space (Fig. 1). This obviated the need for sporadic replenishment of water/nutrients, the fluid parameters of which in microgravity have been extremely difficult to control.

Figure 1. WSU "Nutrient Pack"
1. Polypropylene envelope
2. Gas diffusion membrane
3. Seed support matrix
4. Support column
5. Air phase
6. Germination medium
7. Basic nutrient medium
8. Enriched medium



Using this approach, it was found that *T. aestivum* wheat seedlings in the "nutrient pack" grew very well in space with little differences observed in shoot and root tissue growth/fresh weight over the 10-day flight when compared to the corresponding ground controls. Moreover, electron microscopic examination of cross-sections of serial sections of wheat shoots and roots revealed essentially no differences. That is, the organelles in the cytosol were very similar, and in the root cell walls there were no measurable changes in the cellulose microfibril orientation/cell wall thickness as revealed by freeze-fracture/TEM/SEM analyses (Fig. 2). This latter observation is particularly important since it strongly implies that either the process guiding microfibril orientation is fully sensitive to even a microgravity stimulus, or it occurs independent of the g-force experienced.

**Significance:** This is the first data set that has been obtained whereby excellent growth, relative to the 1 g controls, was attained in space over a relatively "long duration". Importantly, cell wall assembly was not significantly affected; these results are in direct contradiction to previous studies whose plants, it can now be hypothesized, suffered from various stresses in microgravity. Consequently, these results underscore the growing need for development of reliable plant growth systems for the microgravity environment.

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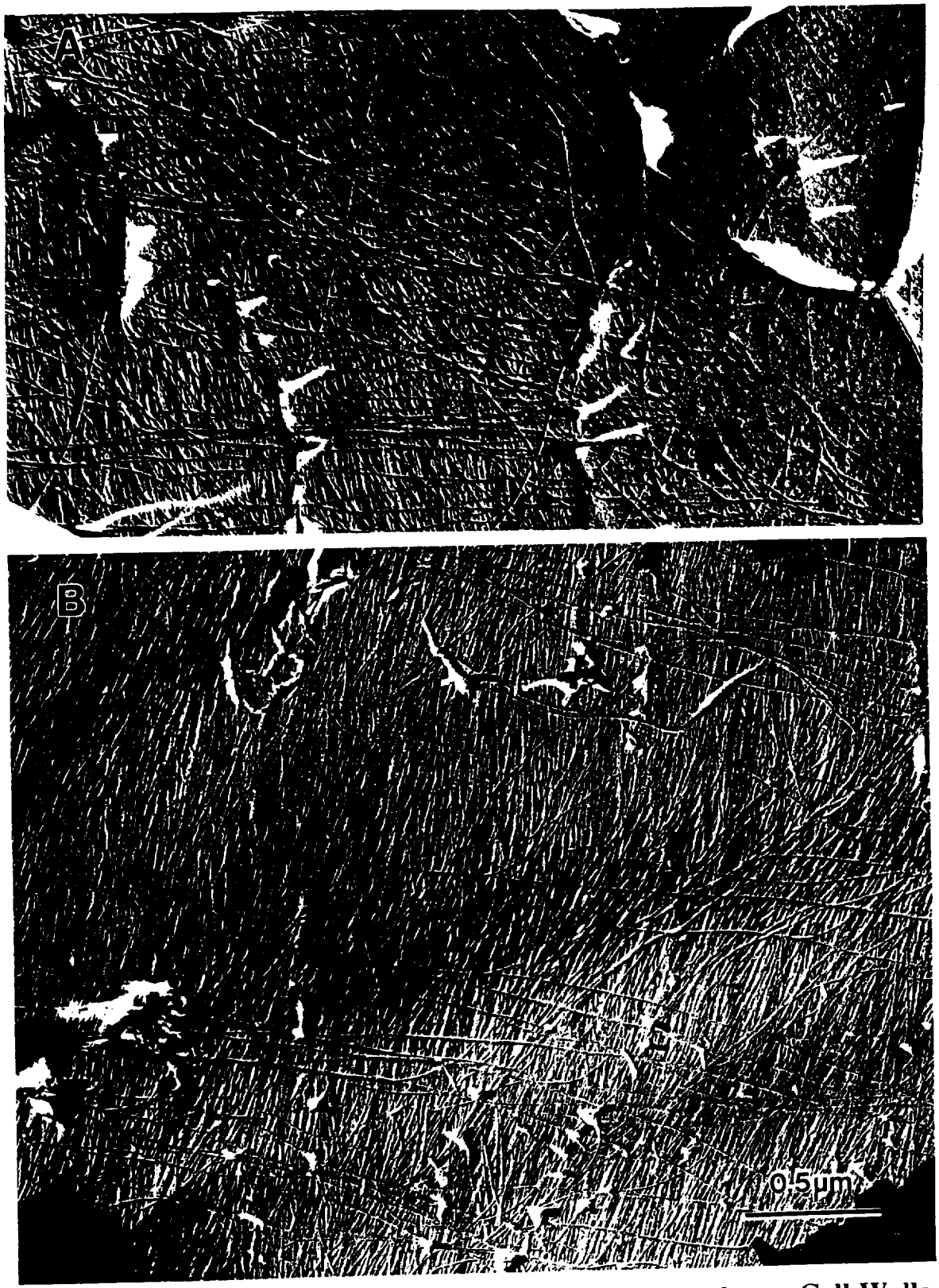


**Microfibril Organization of the Inner Surfaces of Parenchyma Cell Walls in Wheat Primary Roots. A) 1 *g* Control, B) 10 Day Microgravity Exposure**